

AMENDMENTS TO THE CLAIMS

Prior to substantive examination, Applicants have cancelled claims 14 and 15 without prejudice to their subsequent reintroduction into this application or their introduction into a related application. Applicants have amended claims 1-3, 5-7 and 9-13 without any intention of disclaiming equivalents thereof. Applicants have added new claims 16 to 22. This listing of claims will replace all prior versions and listings of claims in the application:

What is claimed is:

1. (currently amended) A method for detecting in a nucleic acid sample the presence or absence of a at least two variant ~~nucleotide in at least two~~ SNP sites nucleotides associated with thrombosis, ~~said SNP sites selected from the group consisting of Factor V Leiden G1691A, Prothrombin (Factor II) G20210A, MTHFR C677T, MTHFR A1298C, Factor XIII G4377T, and tissue factor plasma inhibitor (TFPI) C536T,~~ the method comprising the steps of:

a) amplifying from the sample regions of DNA that include at least two selected nucleotide positions for which variants are known to be associated with thrombosis, ~~containing the at least two SNP sites~~ to form amplified DNA products;

b) hybridizing at least two tagged allele specific extension primers to a complementary target sequence in the amplified DNA products, wherein each tagged allele specific extension primer has a 3'-end hybridizing portion capable of hybridizing to the corresponding amplified DNA, and wherein the 3' end hybridizing ~~portion~~ portions of the at least two tagged allele specific extension primers each comprise a sequence selected from the group consisting of bases from position 25 and up to the 3' terminal nucleotide of SEQ ID NO: 1 to SEQ ID NO: 12, and a 5'-end tag portion complementary to a corresponding ~~probe~~ anti-tag sequence, the terminal nucleotide of the 3' end hybridizing portion being either complementary to a suspected variant nucleotide or to the corresponding wild type nucleotide ~~of the SNP site~~;

c) extending the at least two tagged allele specific extension primers, using labelled nucleotides, if the terminal nucleotide of ~~the~~ each 3' end hybridizing portion is a perfect match to ~~an allele of one of the SNP sites in the~~ the corresponding amplified DNA product products; and

d) hybridizing the at least two tagged allele specific extension primers to ~~the~~ their corresponding ~~probe sequence~~ anti-tag sequences and detecting the presence of labelled extension products.

2. (currently amended) The method of claim 2 1, wherein the 5'-end tag portions of the at least two tagged allele specific primers each comprise ~~comprises~~ a sequence selected from the group consisting of bases 1 to 24 of SEQ ID NO: 1 to SEQ ID NO: 12 and wherein the sequence of each 5'-end tag portion is different from each other 5'-end tag portion.

3. (currently amended) The method of claim 1 wherein the ~~probe~~ anti-tag sequence is coupled to a solid support.

4. (previously presented) The method of claim 3 wherein the solid support is selected from the group consisting of beads, spectrally coded beads, and a chip based microarray.

5. (currently amended) The method of claim 1 wherein the step of amplifying is conducted by PCR using a set of PCR amplification primers, said set comprising at least two pairs of PCR primers selected from the group of pairs consisting of:
SEQ ID NO: 13 and SEQ ID NO: 14, SEQ ID NO: 15 and SEQ ID NO: 16, SEQ ID NO: 17 and SEQ ID NO: 18, SEQ ID NO: 19 and SEQ ID NO: 20, SEQ ID NO: 21 and SEQ ID NO: 22, and SEQ ID NO: 23 and SEQ ID NO: 24, wherein the at least two pairs of PCR primers are selected for their ability to amplify regions of DNA that include sequences to which the selected at least two tagged allele-specific extension primers will hybridize.

6. (currently amended) A method for detecting in a nucleic acid sample the presence or absence of a at least two variant ~~nucleotide in at least two SNP sites~~ nucleotides associated with thrombosis, ~~said SNP sites selected from the group consisting of Factor V Leiden G1691A,~~

~~Prothrombin (Factor II) G20210A, MTHFR C677T, MTHFR A1298C, Factor XIII G4377T, and tissue factor plasma inhibitor (TFPI) C536T,~~ the method comprising the steps of;

a) amplifying from the sample regions of DNA that include at least two selected nucleotide positions for which variants are known to be associated with thrombosis ~~containing the at least two SNP sites~~ to form amplified DNA products;

b) hybridizing at least two tagged allele specific extension primers to a complementary target sequence in the amplified DNA products, wherein the at least two tagged allele-specific extension primers are selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 12, each tagged allele specific extension primer having a 3'-end hybridizing portion capable of hybridizing to the corresponding amplified DNA, and a 5'-end tag portion complementary to a corresponding ~~probe~~ anti-tag sequence, the terminal nucleotide of the 3' end hybridizing portion being either complementary to a suspected variant nucleotide or to the corresponding wild type nucleotide ~~of the SNP site~~;

c) extending the at least two tagged allele specific extension primers, using labelled nucleotides, if the terminal nucleotide of ~~the~~ each 3' end hybridizing portion is a perfect match to ~~an allele of one of the SNP sites in the~~ corresponding amplified DNA ~~product~~ products;

d) hybridizing the at least two tagged allele specific extension primers to ~~the~~ their corresponding ~~probe sequence~~ anti-tag sequences and detecting the presence of labelled extension products.

7. (currently amended) The method of claim 6 wherein the ~~probe~~ anti-tag sequence is coupled to a solid support.

8. (previously presented) The method of claim 7 wherein the solid support is selected from the group consisting of beads, spectrally coded beads, and a chip based microarray.

9. (currently amended) The method of claim 6 wherein the step of amplifying is conducted by PCR using a set of PCR amplification primers, said set comprising at least two pairs of PCR primers selected from the group of pairs consisting of:

SEQ ID NO: 13 and SEQ ID NO: 14, SEQ ID NO: 15 and SEQ ID NO: 16, SEQ ID NO: 17 and SEQ ID NO: 18, SEQ ID NO: 19 and SEQ ID NO: 20, SEQ ID NO: 21 and SEQ ID NO: 22, and SEQ ID NO: 23 and SEQ ID NO: 24, wherein the at least two pairs of PCR primers are selected for their ability to amplify regions of DNA that include sequences to which the selected at least two tagged allele-specific extension primers will hybridize.

10. (currently amended) A kit for use in detecting in a nucleic acid sample the presence or absence of a at least two variant nucleotides ~~nucleotide in at least two~~ SNP sites associated with thrombosis, said SNP sites ~~selected from the group consisting of Factor V Leiden G1691A, Prothrombin (Factor II) G20210A, MTHFR C677T, MTHFR A1298C, Factor XIII G4377T, and tissue factor plasma inhibitor (TFPI) C536T,~~ said kit comprising a set of at least two tagged allele specific extension primers wherein each tagged allele specific extension primer has a 3'-end hybridizing portion including a 3' terminal nucleotide being either complementary to a ~~suspected~~ variant nucleotide known to be associated with thrombosis or to the corresponding wild type nucleotide ~~of one of the SNP sites~~ and a 5'-end tag portion complementary to a corresponding ~~probe~~ anti-tag sequence, and wherein the at least two tagged allele-specific extension primers are selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 12.

11. (currently amended) The kit of claim 10 further comprising a set of PCR amplification primers ~~for amplifying regions of DNA containing the at least two SNP sites,~~ said set comprising at least two pairs of PCR primers selected from the group of pairs consisting of: SEQ ID NO: 13 and SEQ ID NO: 14, SEQ ID NO: 15 and SEQ ID NO: 16, SEQ ID NO: 17 and SEQ ID NO: 18, SEQ ID NO: 19 and SEQ ID NO: 20, SEQ ID NO: 21 and SEQ ID NO: 22, and SEQ ID NO: 23 and SEQ ID NO: 24, wherein the at least two pairs of PCR primers are selected for their ability to amplify regions of DNA that include sequences to which the selected at least two tagged allele-specific extension primers will hybridize.

12. (currently amended) The kit of claim 10 further comprising a set of ~~probes~~ anti-tags, each anti-tag having a sequence complementary to nucleotides 1-24 of the selected at least two tagged allele-specific extension primers.

13. (currently amended) The kit of claim 12 wherein the ~~set of probes~~ anti-tags are coupled to a support.

14 -15. (cancelled)

16. (new) A composition comprising a plurality of polynucleotide primers for use in detecting the presence or absence of variant nucleotides associated with thrombosis, wherein the plurality of primers comprises oligonucleotides having sequences set forth by position 25 to the 3' terminal nucleotide of SEQ ID NOs: 1-12, or the complete complements thereof.

17. (new) The composition of claim 16 wherein the plurality of primers consists of oligonucleotides having sequences set forth by SEQ ID NOs: 1-12 or the complete complements thereof.

18. (new) A combination comprising the composition of claim 17 wherein the plurality of primers consists of SEQ ID NOs: 1-12, and a set of anti-tags, the set of anti-tags having sequences complementary to nucleotides 1-24 of SEQ ID NOs: 1-12.

19. (new) A combination comprising the composition of claim 17 wherein the plurality of primers consists of the complete complements of SEQ ID NOs: 1-12, and a set of anti-tags, the set of anti-tags having sequences complementary to nucleotides 1-24 of the complete complements of SEQ ID NOs: 1-12.

20. (new) The combination of claim 18, wherein each anti-tag is attached to a spectrally coded bead specific for the anti-tag.

21. (new) The combination of claim 19, wherein each anti-tag is attached to a spectrally coded bead specific for each anti-tag.

22. (new) An improved method of simultaneously detecting in a sample the presence or absence of variant nucleotides associated with thrombosis, wherein the improvement comprises simultaneously identifying the presence or absence of variant nucleotides associated with thrombosis via allele specific primer extension using a set of primers having the sequences set forth in SEQ ID NOs: 1-12.